PROPOSAL FOR TOPIC APPROVAL TO BE SUBMITTED TO THE UNIVERSITY OF MUMBAI FOR THE PH.D DEGREE IN THE SUBJECT OF PHARMACOGNOSY.

Title of the topic:	**Formulation and Screening of Cardioprotective Activity of Indian Medicinal Plants.**
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Date: 20/02/2023

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1

INDEX

Sr. No.	Contents	Page No.
1.	Title	1
2.	Aims of study	3
3.	Objectives of Study	3
4.	Study Background (Literature Review) and significance of study	4
5.	International Status	24
6.	National Status	24
7.	Study Hypothesis	26
8.	Study Rationale	26
9.	Research Methodology	27
10.	Statistical Plan	30
11.	Limitations of the Study	31
12.	Expected Outcomes of Study	31
13.	Select Bibliography	32

1. **Title: Formulation and screening of cardioprotective activity of Indian medicinal plants.**

2. **Aim of Study:**

The current project aims to formulate, characterize and evaluate the cardio-protective efficacy of silver nanoparticles (AgNPs) loaded with the extract of polyherbal combination of ***Terminalia arjuna, Terminalia chebula, Trapa bispinosa, Emblica officinale, Sida cordifolia, Withania somnifera, Glycerrhiza glabra, Hemidesmus indicus, Tribulus terrestris, Terminalia belerica, Tinospora cordifolia, Commiphora mukul, and Asphaltum punjabianum.***

In Ayurveda, single or multiple herbs (polyherbal) are involved for the treatment. The Ayurvedic literature Sarangdhar Samhita underlines the concept of polyherbalism to attain greater therapeutic efficacy. Sometime the active phytochemical constituents of single plants are not enough to achieve the desirable therapeutic effects. Hence a combination of multiple herbs in a particular ratio can give an improved therapeutic effect. [(1)]

Research says that COVID-19 victims are at higher risk of incident cardiovascular disease including cerebrovascular disorders, ischemic and non-ischemic heart disease, thromboembolic disease, pericarditis, myocarditis and heart failure.[(2)] In addition, 13 out of 17 COVID-19 patients had myocardial injury, as proved by elevated levels of SGPT & SGOT in the serum.[(3)]

3. **Objectives:**

- Procurement, authentication and processing of plant materials.
- Preliminary physicochemical analysis of powdered crude drugs.
- Preparation of extracts and yield optimization.
- Standardization and preliminary phytochemical analysis of plant extracts.
- Preparation of polyherbal extract in particular combination.
- Standardization, quantification, method development and validation of polyherbal extract
- Synthesis and characterization of silver nanoparticles (AgNPs) loaded with polyherbal extract.
- Evaluation of silver nanoparticles (AgNPs) loaded with polyherbal extract.
- Bio-evaluation of silver nanoparticles (AgNPs) loaded with polyherbal extract.

4. Study Background (Literature Review) and significance of study

Even though there are recent advancements in the field of diagnosis, and management of many CVDs, mortality associated with CVDs remains much higher than cancer across the world. Treatment methods of CVDs include control of blood pressure, cholesterol, weight, diabetes, and physical activity, depression, and selection of ideal medications. Various medications can provide for the secondary prevention of CVDs. These medications include beta-blockers, aspirin, statins, and angiotensin-converting enzyme inhibitors. Apart from these medications, surgical operations, and medical devices are also chosen to treat CVDs, such as balloon angioplasty, heart transplantation, valve repair, coronary artery bypass, and replacement and artificial heart surgeries. The drugs used in these treatments produces the side effects such as constipation, rash, drowsiness, nausea, edema, low blood pressure, renal failure, headache, and hemorrhage. Hence the demand of herbal drugs over conventional and synthetic drugs has been increased. [4]

The most frequent plants used in the herbal formulation and having known cardioprotective activity engaged in this research are *Terminalia arjuna* (Arjuna), shows protective effects against Dox-induced cardiotoxicity,[5] *Terminalia chebula* (Haritaki), has cardioprotective activity in isolated frog heart model,[6] *Trapa bispinosa* (Shringatak) has antioxidant and anti-inflammatory activity,[7] *Emblica officinale* (Amlaki) shows decrease in total serum cholesterol levels,[8] *Sida cordifolia*(Balamool) elevates the antioxidant enzyme superoxide dismutase (SOD) as indication of cardioprotection,[9] *Withania somnifera* (Ashwagandha) shows increase in the relative heart weight and glycogen content of the heart as evidence of cardioprotection,[10] *Glycerrhiza glabra* (Yashtimadhu)-both glycyrrhizin and glycyrrhetinic acid influence cardiac contractility and relaxation,[11] *Hemidesmus indicus* (Ananthmool) increases the vasodilation response,[12] *Tribulus terrestris* (Gokshuru) relaxes smooth muscles and increases angiotensin converting enzyme (ACE) inhibition & hence reduces the hypertension,[13] *Terminalia belerica* (Bibhitaki)lower the levels of lipid and this prevents the development of atherosclerosis and myocardial infarction,[14] *Tinospora cordifolia* (Guduchi)decrease endothelial inflammation, with cardioprotective effect and antioxidant property,[15] *Commiphora mukul* (Guggulu) has a long history in the treatment of cardiovascular diseases including atherosclerosis and hypercholesterolemia,[16] Mineral pitch (Shilajit) has an outstanding cardioprotective effect and it also stops the harmful effects of ISO on heart tissue.[17]

Herbal drugs need a scientific strategy to deliver the components in a sustained manner to increase patient compliance and bypass repeated administration. This can be fulfilled by designing novel drug delivery systems (NDDSs) for herbal constituents. NDDSs not simply reduce the repeated administration to reduce noncompliance, but also help to improve the therapeutic value by reducing toxicity and increasing the bioavailability, and so on. So incorporation of the herbal extracts into novel formulation systems can add some advantages, such as their bulk dosing and less absorption can be overcome which is the biggest problem being faced by the pharmaceutical corporations.[18]

After all, most of the herbal originated drugs possess insoluble character leading to lower bioavailability and increased systemic clearance requiring repeated administration or higher dose, which cause the drug as a poor candidate for therapeutic use. In phyto-formulation research, developing nano dosage forms has lots of advantages for herbal drugs, including enhancement of solubility and bioavailability, protection from toxicity, enhancement of stability, enhancement of pharmacological activity, sustained delivery, improving tissue macrophages distribution, protection from physical and chemical degradation, etc. Hence, the nano-sized drug delivery systems of herbal drugs have a great future for increasing the activity and avoiding problems associated with herbal medicines. Thus, preparation of the nanocarriers as a NDDS in the traditional medicine system is important in treatment of more chronic diseases like CVDs, asthma, diabetes, cancer, etc. [18]

In general, metal nanoparticles comprised of gold, silver, iron, platinum, copper, silica, zinc, and some lanthanides are used as carriers of different biomolecules, specifically drugs, peptides, nucleic acids, and antibodies. It can act as diagnostic and therapeutic agents to various disease models including cardiovascular disease, cancer, microbial infections, and neurodegenerative disease. Because of lots of applications of metal nanoparticles are available in the literature. It was hence thought worthwhile to formulate, characterize and evaluate cardio protective activity of silver nanoparticles loaded with the extracts of above mentioned 13 drugs.

Pharmacognostic accounts of the drugs:

1. ***Terminalia arjuna* (Arjuna):**

(5)

Taxonomical classification:

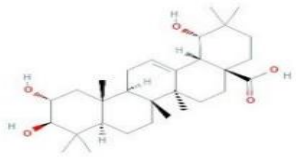
- **Common Name:** Arjuna
- **Kingdom:** Plantae
- **Division:** Magnoliophyta
- **Class:** Magnoliopsida
- **Order:** Myrtales
- **Family:** Combretaceae
- **Genus :** Terminalia

Species: Terminali aarjuna[(5)]

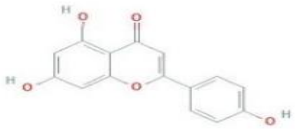
Parts Used: Bark

Phytochemical investigation:

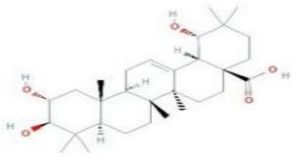
Arjunolic acid, arjunic acid, arjunone, arjungenin, arjunolone and luteolin, ellagic acid, gallic acid, oligomeric proanthocyanidins (opcs), and phytosterols are the major phytoconstituents of Terminalia arjuna that possesses many useful biological properties like cardio protective, antimicrobial, antioxidant, anti-inflammatory, antifeedant, etc. [(5)]



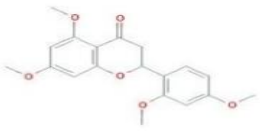
a. Arjunin Acid



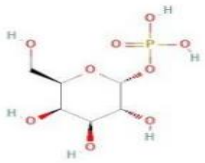
b. Apigenin



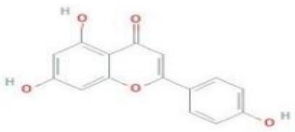
c. Arjunic acid



d. Arjunone



e. Galactose



f. Arjunolic acid

Traditional Medicinal utilities:

The stem bark powder of Arjuna was firstly used by Vegabhatta (वाग्भट) for heart ailments. In Ayurveda, it is mentioned as Hridya (Cardiac tonic). The bark powder of Arjuna was also used for the treatment of kshata (injury or wound), visha (poison), raktavikara (as a styptic), kshaya (emitiated condition), prameha (urinary disorders), medaroga (diabetic issues), vrana (ulcer/wound), etc. [5]

2. ***Terminalia chebula*** **(Haritaki):**

Figure 1: Leaves of *T. chebula.*

Figure 2: Fruits of *T. chebula.* (19)

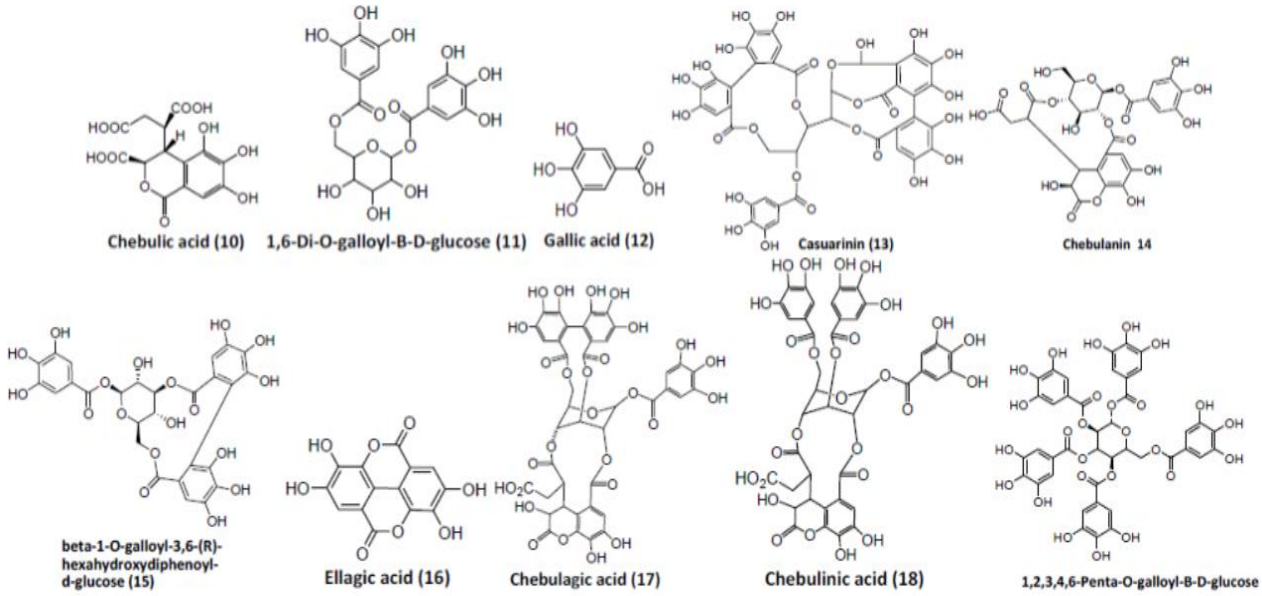
Taxonomical classification:

- **Kingdom:** Plantae
- **Division:** Magnoliophyta
- **Class:** Mangoliopsida
- **Order:** Myrtales
- **Family:** Combretaceae
- **Genus :** Terminalia
- **Species:** Terminalia bellerica [19]

Parts Used: Fruit

Phytochemical investigation:

Total phyto-constituents present in Terminalia chebula are hydrolysable tannins (which may differ from 20-50%) which is responsible for pharmacological activities. Other phytochemials are gallic acid, ellagic acid, chebulinic acid, chebulagic acid, chebulic acid, etc. [20]



Traditional Medicinal utilities:

'Triphala' is commonly used herbal preparation, prepared from fruits of T. chebula, T. bellerica, E. officinalis, and also used as laxative in chronic constipation, food digestive problems (poor digestion and assimilation),detoxifying agent of the colon, and rejuvenator of the body. Triphala is observed as the most useful of all herbal formulations and is prescribed as a cardiotonic and for Candida infection. In Thai traditional medicine it is used as a carminative, astringent and expectorant. It is among the top listed plant in Ayurvedic material medica for the treatment of sore throat, vomiting, asthma, bleeding piles and gout. It is also used as anti-hemorrhagic agent because it provides defence against bleeding. Powdered drug is used for cleansing of teeth with believes to make gums and teeth stronger. [20]

3. *Trapa bispinosa* (Shringatak):

(21)

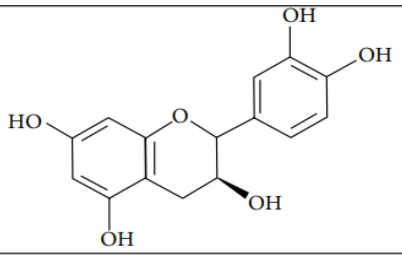
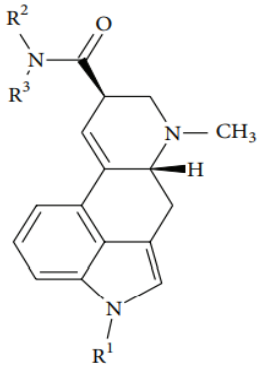
Taxonomical classification:

- **Kingdom:** Plantae
- **Subkingdom:** Tracheobionta
- **Family:** Trapaceae
- **Genus :** Trapa
- **Species:** Trapanatans L. [21]

Parts Used: Fruit

Phytochemical investigation:

Trapa bispinosa contains high quantity of minerals, ions, namely, K, Na, Ca, Zn, and vitamins (A, B1, B2, B3, B5, B6 and C); alkaloids, saponins, phenols, H-donation, flavonoids are reported in *Trapa bispinosa*. [21]

Flavonoids	
Alkaloids	

Traditional Medicinal utilities:

Trapa bispinosa is a crucial plant of Indian Ayurvedic system of medicine which is useful for treatment of stomach, liver, genitourinary system, spleen and kidney. It is astringent, bitter, stomachic, febrifuge, diuretic, and antiseptic. The whole plant is useful in gonorrhea, menorrhagia, and other genital affections. It is also used in dysentery, diarrhea, ulcers, ophthalmopathy, and wounds. These are used in the validated conditions in burning sensation, pitta, dipsia, hemorrhage, dyspepsia, hemoptysis, dysentery, diarrhea, strangely, leprosy, fatigue, intermittent fever, inflammation, erysipelas, urethrorrhea, fractures, lumbago, bronchitis, pharyngitis and general debility, and heart burning and suppressing stomach.[21]

4. ***Emblica officinale*** **(Amlaki):**

(22)

Taxonomical classification:

- **Kingdom :** Plantae
- **Division :** Flowering plant
- **Class :** Magnoliopsida
- **Order :** Malpighiales
- **Family:** Phyllanthaceae
- **Tribe :** Phyllantheae
- **Subtribe:** Fluegginae (22)

Parts Used: Fruit

Phytochemical investigation:

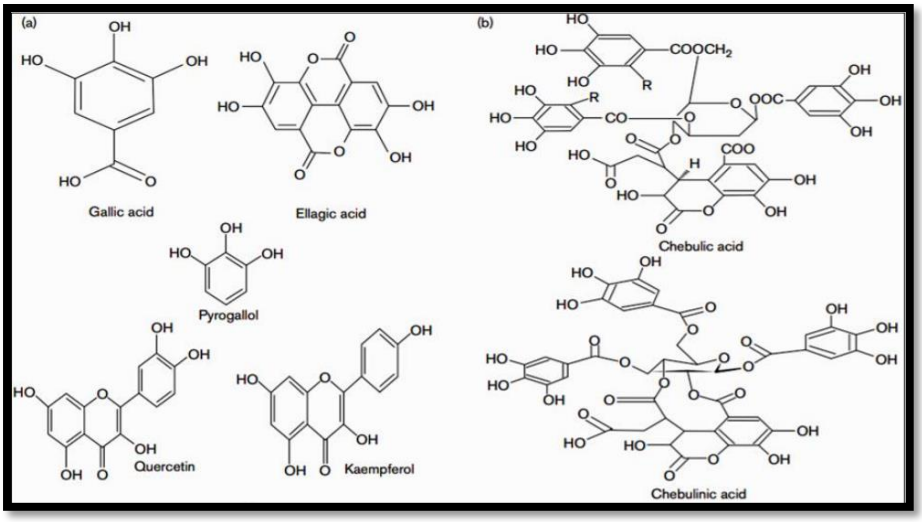
The chemical constituents present in Phyllanthus emblica are alkaloids, vitamins, amino acids, organic acids, phenolic compounds, hydrolysable tannins, flavonoids, carbohydrates.

These constituents can be further described as, it contains alkaloids such as Phyllantine, Phyllembein, and Phyllantidine.

The vitamin present is Ascorbic acid. The amino acids present are Glutamic acid, Proline, Aspartic acid, Alanine, Cystein, Lysine. The organic acid present is Citric acid.

The phenolic compounds present are Gallic acid, Ellagic acid, Methyl gallate, and Trigallayl glucose.

The hydrolysable tannins present are Emblicanin A and B, Pedunculagin, Ellagotannin, Geraniin (Dehydroellagitannin), Corilagin (Ellagitannin), Chebulagic acid (Benzopyran tannin), Chebulinic acid (Ellagitannin), Punigluconin. The carbohydrate present is Pectin. The flavonoids present are Quercetin and Kaempferol. (22)



Traditional Medicinal utilities:

In Ayurveda, amla is known to be a potent rasayana (rejuvenator) and used in stopping the degenerative and senescence process, to promote benefits of eye, stimulate hair growth, longevity, enhance digestion, to treat constipation, enliven the body, enhance the intellect, strengthen the heart, reduce fever, purify the blood and reduce cough alleviate asthma. [22]

5. ***Sida cordifolia*** **(Balamool):**

[23]

Taxonomical classification:

- **Kingdom:** Plantae
- **Division:** Angiospermae
- **Class:** Eudicots
- **Order :** Malvales
- **Family:** Malvaceae
- **Genus :** Sida
- **Species:** Sida cordifolia [24]

Parts Used: Root

Phytochemical investigation:

Chemical constituents of Sida cordifolia are ephedrine, pseudoephedrine, sterculic, malvalic and coronaric acid, fatty acids, saponine, beta phenethylamine, hypaphorine, ecdysterone, indole alkaloids [24]

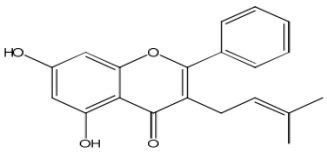


Fig. 1:- 5, 7-dihydroxy-3-isoprenyl flavones.

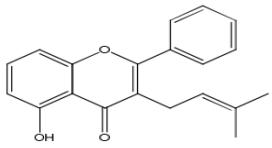


Fig. 2:- 5-hydroxy-3-isoprenyl flavones.

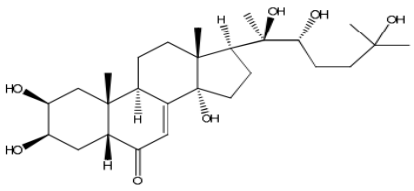


Fig. 3:- Structure of Ecdysterone.

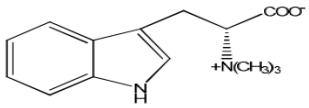


Fig. 4:- Structure of hypaphorine

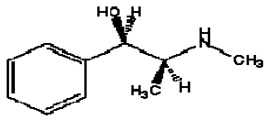


Fig 5:- Structure of ephedrine

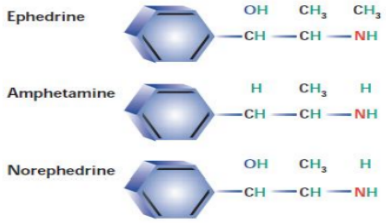


Fig 6:- Ephedrine Derivatives

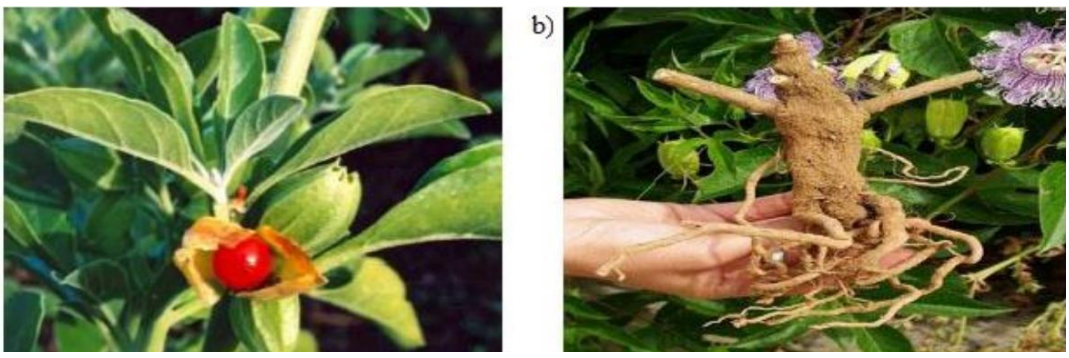
(24)

Traditional Medicinal utilities:

It owns a long history of its use in Ayurveda and rural area especially for medicinal properties. Used as folk medicine in India since ancient time. According to Ayurveda, the plant is tonic, emollient, astringent, aphrodisiac and useful in treatment of respiratory system related problem. Bark is known for cooling. It is useful in fever, blood, urinary system related troubles, throat, piles, insanity, phthisis, etc. The plant is anti-inflammatory, analgesic and tonic. It also affects the central nervous system and gives relief from anxiety. Extract is consumed to reduce body weight. It normalizes the blood pressure and improves the cardiac irregularity. [24]

6. *Withania somnifera* (Ashwagandha):

a) b)



(25)

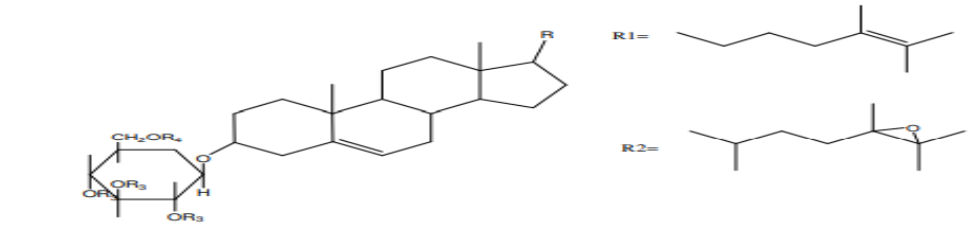
Taxonomical classification:

- **Kingdom:** Plantae, Plants
- **Subkingdom:** Tracheobionta
- **Super division:** Spermatophyta
- **Division:** Angiosperma
- **Class:** Dicotyledons
- **Order :** Tubiflorae
- **Family:** Solanaceae
- **Genus :** Withania
- **Species:** Somnifera Dunal [26]

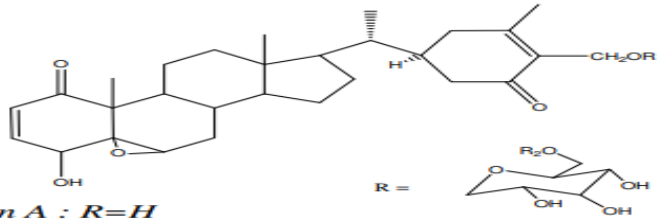
Parts Used: Root

Phytochemical investigation:

The biologically active chemical constituents of WS are alkaloids (anahygrine, ashwagandhine, tropine, cuscohygrine, etc), steroidal compounds, includesergostane type withaferin A, steroidallactones, withanolides A-y, withasomidienone, withasomniferin-A, withasomniferols A-C, withanone etc. Plant also contain chemical constituents like withaniol, hantreacotane, ducitol, starch, reducing sugar, a variety of amino acids including aspartic acid, alanine, proline, tyrosine, glycine, tryptophan, glutamic acid, cystine, and high amount of iron. [26]



Sitoindoside VII : R=R₁ : R₃=H : R₄ = Palmitoyl
Sitoindoside VIII : R=R₂ : R₃=H : R₄



Withaferin A : R=H
Sitoindoside IX: R₁ = D-glucoside; R₂=H
Sitoindoside X: R₁ = D-glucoside; R₂=Palmitoyl = Palmitoyl (26)

Traditional Medicinal utilities:

Different parts of the plant, such as roots, leaves, bark, flowers, and stem, are traditionally used to cure heart problems, liver disorders, pain, respiratory infections, fever, ulcers, wounds, and sex-related diseases. Curing potential of WS in ancient systems of medicines (Ayurvedia and Unani) owed to bioactives such as steroids, alkaloids, flavonoids, phenolics, etc. [25]

7. ***Glycerrhiza glabra*** **(Yashtimadhu):**

(27)

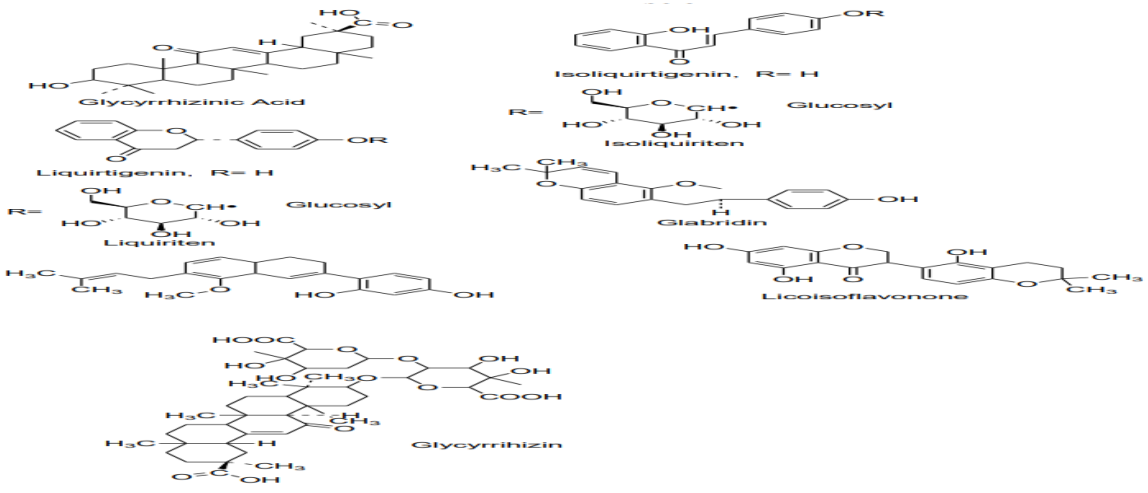
Taxonomical classification:

- **Kingdom :** Plantae
- **Division :** Phanerogams
- **Subdivision :** Angiospermae
- **Class :** Dicots
- **Subclass :** Polypetalae
- **Order :** Calciflorae
- **Family:** Leguminosae
- **Subfamily:** Papilionaceae
- **Genus:** Glycyrrhiza
- **Species:** Glabra [28]

Parts Used: Root

Phytochemical investigation:

Chemical constituent is Glycyrrhizin – a saponin derivative. It is a triterpenoid saponin which is a mixture of calcicum salts and potassium of glycyrrhizic acid and it is 60 times sweeter than sugarcane. Flavonoid rich fractions include liquiritin, isoliquiritin, neoliquiritin, liquiritigenin and rhamnoliquirilin. Liquiritin is a chalcone derivative which gives yellow color to the drug. The root contains glycyrrhizin, glycyrrhiziniz acid, sugar, saponins, starch, aspargin, gum, resin, mucilage, magnesium and calcium salts. The bark contains small amount of tannins. Isoflavonoid derivatives such as galbrene, glabridin, shinpterocarpin, glabrone, licoisoflavones A and B, glyzarin, formononetin and kumatakenin are also present. [28]



(29)

Traditional Medicinal utilities:

Traditionally, it has been in use as a flavouring agent and sweetener in several foods and medicines. Plant has been in use since ancient times as a prophylaxis in duodenal and gastric ulcers for to its anti-ulcer activity. Used in treatment of cough because of its expectorant and antitussive properties. It is also used as a laxative, antiasthmatic, galactogogue, anti-viral agent and antacid. It is useful as one of the major component in medicinal oils used for treatment of paralysis, rheumatism and epilepsy. Liquoricealso accelerates the action of senna. Liquid extract of liquorice is used in the treatment of peptic ulcer. [28]

8. ***Hemidesmus indicus*** **(Ananthmool):**

(30)

Taxonomical classification:

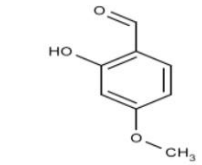
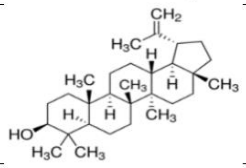
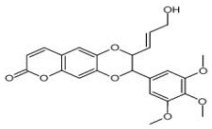
- **Kingdom :** Plantae
- **Phylum :** Tracheophyta
- **Class :** Magnoliopsida
- **Order :** Gentianales
- **Family:** Asclepiadaceae
- **Genus :** Hemidesmus
- **Species :** Indicus [30]

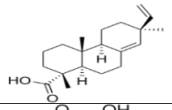
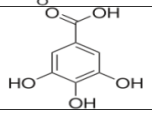
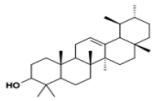
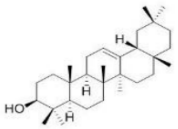
Parts Used: Root

Phytochemical investigation:

Many phytocompounds are isolated from various parts, like roots is a source of several phytocompounds with therapeutic values. It possesses resin, hemidesmol, glucoside, and tannin; α- and β- amyrins; β-sitosterol, lupeol acetate, β-amyrinacetate, hexa-lupeol 1- octacosonal, Triconate acid, steroid, lupeol, flavonoid, terpenoid, and saponin. It also contains 80% of crystalline material such as glucose hemidesmol, glucoside, 2- hydroxy-4-methoxy benzaldehyde, sterol, resin acid and tannins. Stem contains glycosides like indicine and hemidine. Chloroform and alcohol extract of the stem yield pregnane emidine,glycosidesand hemidescine. Leafcontains 2.5% of tannins. hemidesmin 1, hemidesmin 2 and

Coumarinolignoids hemidesminine were also isolated from leaves. Coumarinolignoids is new and rare group of naturally occurring compound, with antihepatotoxic and cytotoxic properties. Flower contains hyperoxide, glycosides, rutinand Isoquercetin. (30)

2-hydroxy, 4-methoxybenzaldehyde (2H4MB)	
Lupeol	
Hemidesminine	

Resin acid	
Tannins	
α-amyrin	 α-Amyrin
β-amyrin	

(30)

Traditional Medicinal utilities:

The plant roots were used as antidiarrheal, antipyretic and blood purifier. They are used for the treatment of blood diseases, diarrhoeaand dysentery, skin diseases, respiratory disorders, leprosy, itching, leukoderma, bronchitis, syphilis, asthma, epileptic fits in children, eye diseases, burning sensation, lack of appetite, rheumatism, urinary and kidney disorders. (30)

9. *Tribulus terrestris* (Gokshuru):

Figure 1: Whole plant of *tribulus terrestris*

Figure 2: Fruit of *tribulus terrestris* plant (31)

Taxonomical classification:

- **Kingdom :** Plantae
- **Division :** Phanerogams
- **Subdivision :** Angiospermae
- **Class :** Dicotyledonae
- **Subclass :** Polypetalae
- **Series :** Disciflorae
- **Order :** Giraniales
- **Family:** Zygophyllaceae
- **Genus :** Tribulus
- **Species :** Terrestris Linn (31)

Parts Used: Fruit

Phytochemical investigation:

TT revealed the presence of flavonoids, saponins, alkaloids, glycosides, and tannins. As per the literature data, the saponin composition and the saponin content can differ based on the geographic regions. Other components like furostanol and spirostanol saponins of tigogenin, gitogenin, neotigogenin, neogitogenin, hecogenin, diosgenin, ruscogenin, chlorogenin and sarsasapogenin types are frequently found in this plant. Major components present are furostanol glycosides including protogracillinand protodioscin, of which protodioscin saponinis present in large amount and spirostanol glycosides are present in small quantities. (31)

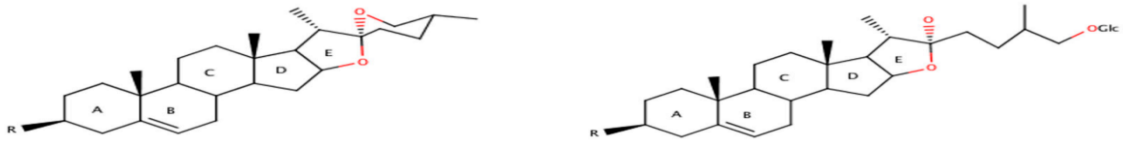


Figure 1. Spirostanol **(left)** and furostanol **(right)** saponins.

Traditional Medicinal utilities:

TT is used in folk medicines as a tonic, palliative, aphrodisiac, astringent, antihypertensive, stomachic, diuretic, lithotriptic, and urinary disinfectant. The dried fruit is very effective in utmost genitourinary tract disorders. It is a vital constituent of Gokshuradi Guggul, a potent Ayurvedic medicine useful to support proper working of the genitourinary tract and to eliminate the urinary stones. TT has been used for centuries in Ayurveda to treat venereal diseases, impotence, and sexual debility. The Ayurvedic Pharmacopoeia of India defines cardiotonic properties to the root and fruit. In traditional Chinese medicine, the fruits were used for treatment of edema, eye trouble, abdominal distension, morbid leukorrhea, emission, and sexual dysfunction. (31)

10. *Terminalia belerica* (Bibhitaki):

(32)

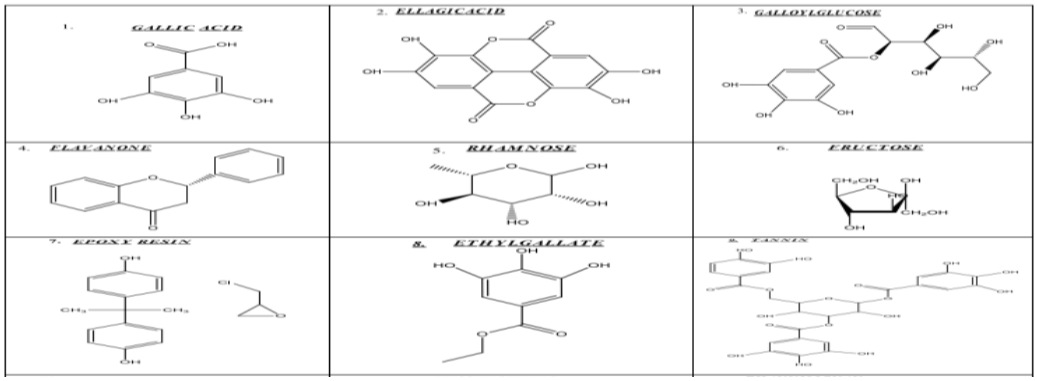
Taxonomical classification:

- **Family Name :** Combretaceae
- **Kingdom :** Plantae
- **Division :** Magnoliophyta
- **Class :** Magnoliopsida
- **Order :** Myrtales
- **Family:** Combretaceae
- **Genus :** Terminalia
- **Species :** Belerica [32]

Parts Used: Fruit

Phytochemical investigation:

The chief chemical constituents of *Terminalia belerica* are gallic acid, ellagic acid, galloylglucose, flavones, fructos, rhamnose, epoxy resin, ethylgallate, tannins, etc. [32]



(32)

Traditional Medicinal utilities:

In Ayurveda, the drug T. belerica is used as an expectorant. It is an important part of Ayurvedic laxative, anthelmic, astringent, and antipyretic which is used in hepatitis, piles, bronchitis, coughs, eye disease such as corneal opacity, myopia, etc. It is also used as hair tonics. It has rejuvenative, lithotriptic and antibacterial properties. The fruits are used in digestive, aperients, astringents, narcotic, stypic. Unripe fruit is used as mild laxative and ripe fruit is used as an astringent, whereas decoction of the fruit is used for treating cough. [32]

11. *Tinospora cordifolia* (Guduchi):

(33)

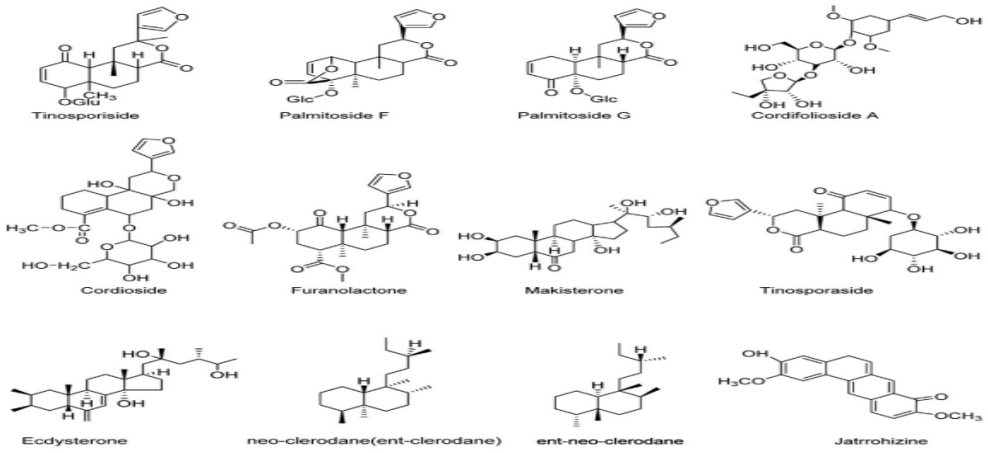
Taxonomical classification:

- **Kingdom :** Plantae
- **Division :** Magnoliophyta
- **Class :** Magnoliopsida
- **Order :** Ranunculales
- **Family:** Menispermaceae
- **Genus :** Tinospora
- **Species :** Cordifolia [15]

Parts Used: Stem

Phytochemical investigation:

The chemical constituents of T. cordifolia belong to different classes such as glycosides, alkaloids, steroids, aliphatic compounds, phenolics, polysaccharides. Leaves are rich in protein (11.2%), phosphorus and calcium. The stem contains amritoside A, B, C, and D. The structure of the active chemical constituent of Tinospora cordifolia has been depicted below. [33]



(33)

Traditional Medicinal utilities:

The plant is commonly used as traditional ayurvedic medicine and has several therapeutic properties such as rheumatism, jaundice, urinary disorder, diabetes, anemia, skin diseases, inflammation, anti-periodic, allergic condition, radioprotective properties, etc. The root of T. cordifolia is used as powerful emetic and for bowel obstruction. Starch of this plant serves a beneficial household remedy for relieves burning sensation, chronic fever, increases energy and appetite. It is also useful in the treatment of heart diseases, helminthiasis, leprosy, support the immune system, rheumatoid arthritis, the body's resistance to infections, supports standard white blood cell structure, function, and levels. It also helps in digestive condition such as hyperacidity, worm infestations, colitis, loss of appetite, excessive thirst, abdominal pain, and vomiting, and even liver disorder (hepatitis). [33]

12. *Commiphora mukul* (Guggulu):

Fig. 1: Leaves, stem, fruits and gum resin of *Commiphora mukul* (34)

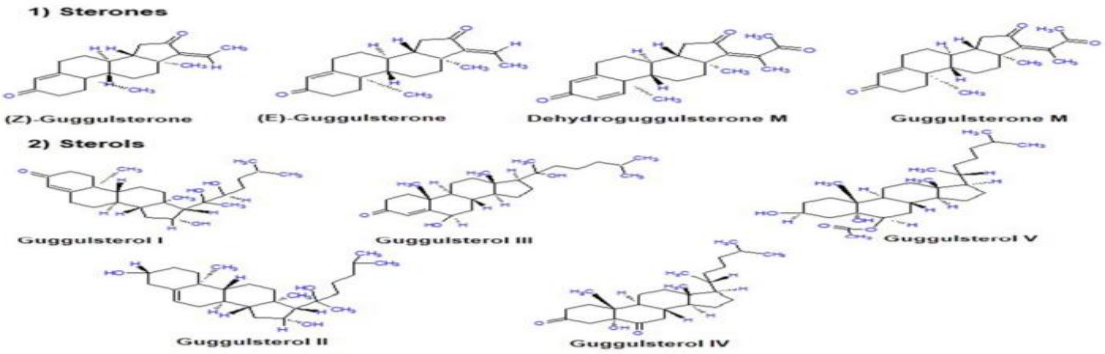
Taxonomical classification:

- **Kingdom:** Plantae
- **Subkingdom:** Tracheobionta
- **Superdivision :** Spermatophyta
- **Division:** Magnoliophyta
- **Class:** Eudicots
- **Subclass:** Rosidae
- **Order :** Sapindales
- **Family:** Burseraceae
- **Genus :** Commiphora
- **Species:** Mukul or wightii (34)

Parts Used: Resin

Phytochemical investigation:

It contains mixture of gum, minerals, sterols (Guggulsterol -I,-II,- III,-IV,-V), terpenes, essential oils, sterones (Z-, E-, M-guggulsterone, and dehydro guggulsterone-M), lignans, ferrulates and flavanones. The ethyl acetate soluble fraction which is guggulipid, consists of various bioactive components like diterpenoids, triterpenoids, lignans, steroidsand fatty tetrol esters. (35)



(35)

Traditional Medicinal utilities:

The oleo-resin-gum is bitter, aromatic, acrid astringent, digestive, antiseptic, anthelmintic, anti-inflammatory, liver tonic, stimulant, rejuvenating, diuretic and general tonic and useful in vitiated conditions of vata, facial paralysis, sciatica, leprosy, cough, asthma, bronchitis, leucodermaand hepatic disorders. The gum is famous for its anti rheumatic and anti-

inflammatory actions. In India the gum resin has been used in treatment of various types of arthritis. Ayurvedic physicians mostly used guggul gum in treatment of arthritis and related conditions for centuries. Powder of bark is taken orally with water to cure cough and cold. Inhalation of the fumes of gum resin cures fever, nasal congestion, bronchitis, laryngitis and phthisis. (34)

13. Asphaltum Punjabianum (Mineral pitch, Shilajit):

(36)

Description: Shilajit is a multi-component natural occurring mineral substance used in Ayurveda and Siddha systems of medicine.

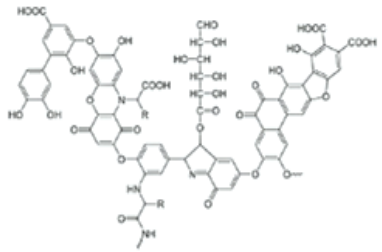
Common names: Mineral pitch, vegetable asphalt, bitumen , Jew's pitch,; Silajatu, mumiyo

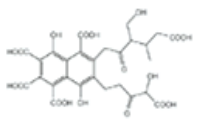
There is no taxonomic classification for a drug Shilajit. It is simply listed as a "drug of mineral origin" in the Ayurvedic Formulary and many literatures. Research at Banaras Hindu University (BHU) in India proves via chemical analysis that Shilajit is the result of the humification of resin and latex bearing plants including *Euphorbia royleana* and *Trifolium repens*. (36)

Phytochemical investigation:

Shilajit contains resins, benzoic acid, hippuric acid, fulvic acid, humic acid.

Minerals: silica, iron, antimony, calcium, copper, lithium, magnesium, manganese, molybdenum, phosphorus, sodium, strontium, zinc. (36)


Humic acid molecule


Fulvic acid molecule

Traditional Medicinal utilities:

It is referred as 'rasayana' / 'rasayanam' in Ayurveda and Siddha literature which means rejuvenator because it prevents or stops ailment and enhances the quality of life. Shilajit has been used in cardiac, asthma, nervous, diabetic, immune and joint disorders. [36]

PHARMACOLOGICAL PROFILES OF THE DRUGS:

Some reports revealing the pharmacological actions of drugs are summarized in table below.

Plant part	Constituents/Extracts	Model and experimental details	Activity	Ref.
***Terminalia arjuna* (Arjuna)**				
Bark	Ethanolic extract	Doxorubicin (Dox)- induced model of male wistar rats	Lowered the total cholesterol, triglycerides and LDL	5
***Terminalia chebula* (Haritaki)**				
Stem bark	Aqueous extract	Isolated frog heart perfusion technique (IFHP)	Cardiotonic effect	6
***Trapa bispinosa* (Shringatak)**				
Fruit	Ethanolic extract	Mice	Antioxidant activity	7
***Emblica officinale* (Amlaki)**				
Fruit	Ethanolic extract	Wistar rats	Cardioprotective efficacy	37
***Sida cordifolia* (Balamool)**				
Leaves	Hydroalcoholic extract	Biomarkers (lactate dehydrogenase and creatine kinase isoenzyme) and antioxidants (superoxide dismutase assay and CAT)	Myocardial injury and antioxidant	9
***Withania somnifera* (Ashwagandha)**				
Root	Root powder	Isoproterenol-induced	Cardioprotective and	10

		myocardial infarctions	anti-oxidant activities	
Root	Hydro-alcoholic extract	Wistar rats	Anti-apoptotic activity and cardioprotective effect	10
***Glycerrhiza glabra* (Yashtimadhu)**				
Root	Ethanolic extract	Ischemia–reperfusion induced injury in rats	Cardioprotective potential	38
***Hemidesmus indicus* (Ananthmool)**				
Root	Ethanolic extract	Langendorff-perfused rat hearts	Cardioprotective effects (ischemia)	39
***Tribulus terrestris* (Gokshuru)**				
Fruit	Ethanolic extract	Cardiac ischemia in rats model	Anti-ischemic cardioprotection	40
***Terminalia belerica* (Bibhitaki)**				
Fruit	Ethanolic extract	Level of lipids detection in rabbits	Hypercholesterolemic effect	41
***Tinospora cordifolia* (Guduchi)**				
Bark	Methanolic extract	Serum marker enzymes (creatine kinase and lactate dehydrogenase)	Cardioprotective effect	42
***Commiphora mukul* (Guggulu)**				
Gum	Ethanolicextrac	Isoprenaline-induced myocardial necrosis in rats	Cardioprotective effect	43
Asphaltum Punjabianum (Mineral pitch, Shilajit)				
Resin	Ethanolic extract	Destructive effects of isoproterenol in rats	Cardioprotective effect	36

5. International Status:

- Mohamed N. A. from Qatar University, Doha along with Marei I from Imperial College, London and other authors reported that nanotechnologies have recognized potential applicability in managing chronic diseases in the past few years, especially CVDs. [44]
- Hesari M. from Kermanshah University of Medical Sciences, Iran along with other authors reported in a review article that " in the context of CVDs prevention and/or treatment, available data suggests that natural-based nano formulations are considerably efficient, alone or in blend with other herbal/synthetic medicines". [45]
- Abert H A from Modibbo Adama University, Nigeria along with other authors demonstrated that iron oxide nanoparticles of a herbal extract were effective in ameliorating Triton X-100-induced atherosclerosis in rats, suggesting its cardioprotective action. [46]
- Kiani B H from Islamabad, Pakistan alongwith other authors have reported preparation of zinc nanoparticles of *Withania somnifera*.[48] Wang R at college of life sciences, Korea along with other authors have formulated gold nanoparticles of *Phyllanthus emblica.*[50] Preparation of mucoadhesive nanoparticles of *Glycerrhiza glabra* has been reported by Roque L at Lisboa, Portugal and other authors. [49]

Hence, it can be seen that there has been research conducted internationally on herbal based nanoparticles and their cardioprotective action. But any of them does not match with the aim of this research (Silver nanoparticles loaded with polyherbal extract).

6. National Status

- Teja Parusu Kavya from National Institute of Pharmaceutical Education and Research-Ahmedabad (NIPER-A) alongwith other authors reported that herbal nanomedicines are safer, have higher bioavailability, and have enhanced therapeutic value than conventional herbal and synthetic drugs. [50]
- Vikas Murkute from AISSMS College of Pharmacy, Pune along with other authors reported that "Instead of using monotherapy, combination therapy or polyherbal combination can be a better option to manage cardiovascular disease". [51]

- Balliah R from Department of Biochemistry, PSG College of Arts and Science, Coimbatore, reported that in isoproterenol induced myocardial infarction study in Wistar albinorats, polyherbal crude extract was shown to exhibit the cardioprotective activity. [52]
- Pallela P N at advanced analytical laboratory, Vishakhapatnam along with other authors reported formulating iron nanoparticles of *Sida cordifolia*. [54] Anke Gowda V M at Bangalore Institute of Technology along with other authors reported formulating silver nanoparticles of *T. chebula* fruit extract. [55] Ahmed Q at central university of Rajasthan, Ajmer along with other authors have formulated silver nanoparticles of *T. arjuna* bark extract. [55]
- Brito Raj S at Sri Venkateswara College of Pharmacy, Chittoor, Andhra Pradesh have reported synthesis of silver nanoparticles from a polyherbal formulation. [56] J. Sasidharan at Trichy along with other authors have reported formulating silver and iron nanoparticles from a polyherbal formulation. [57] Hemalakshmi M. and P. Chitra at Sri Ramakrishna college of Arts and Science for Women, affiliated to Bharathiar University Coimbatore, Tamil Nadu have reported synthesis of silver nanoparticles using polyherbal formulation. [58] Eswaran A at Department of Biochemistry, Periyar University, Salem, Tamil Nadu, India along with other authors have reported synthesis of silver nanoparticles from polyherbal formulation. [59]

Hence, it can be seen that there has been research conducted in India on formulating herbal as well as polyherbal nanoparticles. Polyherbal combination is suggested to be a better option than monotherapy in managing cardiovascular disease.

7. Study Hypothesis

Although there are modern advancements in the field of diagnosis and management of many CVDs, death associated with CVDs remains much higher all over the world. Even with the recent progress in research and therapy, cardiovascular diseases are the major common cause of death globally, hence new approaches are required. Targeted therapies and advanced diagnostic tools and involvement of innovative and alternative methods are lacking. Herbal drugs have earned much attention as they slow down the progression of CVDs and are economically attractive. Natural products in CVD medicine have multi-targeted effects with lesser side effects than synthetic drugs. This can be overcome by using standardized extracts and maintaining the

quality control of the raw materials. Presently, nanotechnology is playing a crucial role in the area of CVDs with an opportunity to achieve effective treatment, and less adverse effects on non-target tissues. Smaller in size allows them an advantage to travel intravascular or intracavity actively for drug delivery. Polyphenols and antioxidants available in some plants have significant cardio-protective activity. Hence with this hypothesis is thought valuable to synthesize, characterize and evaluate the cardio protective activity of AgNPs of the herbal extracts of earlier discussed 13 drugs which are constituents of Collasyn tablet.

A study of such kind on polyherbal nanoparticles in management of CVD has not been reported to the best of my knowledge after screening the literature. Therefore if synthesis and stability of silver nanoparticles loaded with polyherbal extract (prepared by taking the same quantity of drugs as in Collasyn tablets) is not achieved, it is thought important to formulate, characterize and evaluate the cardioprotective activity of silver nanoparticles loaded with two or three major herb extracts as in Collasyn tablets or a polyherbal phytosome will be prepared.

8. Study Rationale

Herbal drugs or herbal formulations have always been used as a best alternative against the conventional treatments, but there are some downsides for the herbal remedies too which involve the non-specificity for the target and bulk dosing. It has also been reported that the nano-sized drug delivery system can cover the drawbacks associated with the conventional therapy and traditional drug delivery systems, as well as their non-specificity, severe side effects and damage to the normal cells. Modification of physicochemical properties of nanoparticles such as size, shapeand surface modifications can extraordinarily change its pharmacokinetic and pharmacodynamic data and will provide stronger treatment strategy. Nanotechnology has gained increased significance for delivering the therapeutic agents effectively to the cardiovascular system. Hence, the present study will establish a scientific data for use and emphasis on the synthesis of the AgNPs nanoparticles containing the extracts of earlier discussed 13 drugs. The bio evaluation of the cardio protection activity will discuss the use of these traditional herbs for cardio protection.

9. Research Methodology

9.1. Procurement, authentication of the crude drug

Bark of *Terminalia arjuna,* fruits of *Terminalia chebula*, fruits of *Trapa bispinosa,* fruits of *Phyllanthus emblica* , roots of *Sida cordifolia*, roots of *Withania somnifera*, roots of *Glycerrhiza glabra*, roots of *Hemidesmus indicus,* fruits of *Tribulus terrestris,* fruits of *Terminalia bellerica,* roots of *Tinospora cordifolia,* gum of *Commiphora mukul,* mineral pitch (Shilajit) would be obtained from Dr. Palep's research laboratory as gift samples and authenticated on the basis of microscopic and morphological analysis. Respective parts will be dried and powdered.

9.2. Proximate analysis

The dried crude drug powders would be evaluated for parameters like acid insoluble ash, total ash, moisture content, foreign, matter, extractive values. The result will be compared with the standard Indian Herbal Pharmacopoeia.

9.3. Preparation of extracts and yield optimization

Each powdered crude drug will be extracted using different solvents. Solvents will be selected by yield optimization. Plant extracts will undergo ultrasonication. Then filtered and concentrated by evaporating it in a porcelain dish on electric water bath at 40 °C to remove solvent.

9.4. Standardization and preliminary phytochemical analysis of plant extracts

Prepared extracts will be subjected to preliminary phytochemical analysis using standard tests to detect various types of phytoconstituents. The marker compounds will be utilized for standardizing plant extracts. TLC and UV studies will be done to confirm the presence of marker compounds in the plant extracts. Whichever extract shows maximum concentration of active constituents will be taken for further studies.

9.5 Preparation of polyherbal extract

By taking same quantities of plant extracts as in Collasyn tablet, marketed by Dr.Palep research laboratories, a polyherbal extract will be prepared.

9.6 Standardization, quantification, method development and validation of polyherbal extract using marker compounds

Standardization of polyherbal extract will be done by using UV and TLC. Marker compounds will be used for standardization. TLC and HPLC studies will be done to decide which marker compound needs to be used. Mobile phase would be chosen depending upon the nature of the marker compound. Subsequent quantification of polyherbal extract will be done by HPLC. The

analytical method development would be done using HPLC for the simultaneous estimation of the active biomarkers in the formulation.

9.6.1 Validation of the analytical method

This would be done according to ICH guidelines. The parameters include accuracy, precision, limit of detection, limit of quantification, linearity, range, and robustness.

9.7. Synthesis and characterization of polyherbal loaded silver nanoparticles (AgNPs)

Polyherbal loaded silver nanoparticles (AgNPs) will be synthesized by using conventional methods and by doing optimization. The characterization of the AgNPs will be carried out using UV-vis spectroscopy, X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and transmission electron microscopy (TEM).

9.8. Evaluation of silver nanoparticles (AgNPs) loaded with polyherbal extract

Prepared nanoparticles would be evaluated for parameters like yield of Nanoparticles, Drug Content / Surface entrapment / Drug entrapment, Particle Size and Zeta Potential, surface morphology, Polydispersity index, In-vitro release study, Stability study of nanoparticles.

9.9. Bio-evaluation of silver nanoparticles (AgNPs) loaded with polyherbal extract for cardioprotective activity

9.9.1. In vitro study – Cell line study

Tryptan Blue exclusion test of cell viability: Different quantities of the silver nanoparticles loaded with polyherbal extract and polyherbal extract will undergo Trypan Blue exclusion test of cell viability using H9c2-Rat Cardiomyocyte cell line.

Cells will be pre-treated with various concentrations of silver nanoparticles of polyherbal extract and polyherbal extract prior to the induction of ischemia. To induce ischemia, the cells will be maintained in an ischemic buffer (composition in mM -137 NaCl, 12 KCl, 0.5 $MgCl_2$, 0.9 $CaCl_2$, 20 HEPES, 20 2-deoxy-d-glucose, pH-6.2) at 37°C with 0.1% O_2, 5% CO_2, and 95% N_2 in a hypoxia incubator for 1hour. The analysis will be estimated by cell viability assay using Trypan Blue dye exclusion method and a microscopic monitoring of the morphology of the cells.

Negative control (medium with cells but without the experimental drug/compound) and Positive control (Medium with cells and Cyclosporin A – 1 micromole) followed by Ischemic buffer-1hr as well as Treatment control (Cells pre-treated with various concentrations of silver nanoparticles of polyherbal extract and polyherbal extract without ischaemic buffer).

The percentage viability will be plotted against the quantity of formulation used. The percentage of viable cells will be calculated as follows:

% viability = Number of viable cells / Total number of cells X 100

ATP, which is a mitochondrial function parameter will be measured in cells.

9.9.2. In vivo study - Animal study

The cardioprotective effects will be evaluated in isoproterenol induced cardiotoxicity (myocardial ischemia) in rats. Silver nanoparticles loaded with polyherbal extract and polyherbal extract will be tested in this study. Male wistar albino 10-12 weeks old rats weighing 180 g- 230 g will be used in study. Animals will be divided in groups of 6 rats each.

Group I – Healthy control (orally administered feed +water)

Group II – Disease (Ischaemic) control (Isoproterenol)

Group III – Standard control -propranalol + isoproterenol

Group IV – Treatment control - polyherbal formulation

Group V- Treatment control - Silver nanoparticles loaded with Polyherbal extract

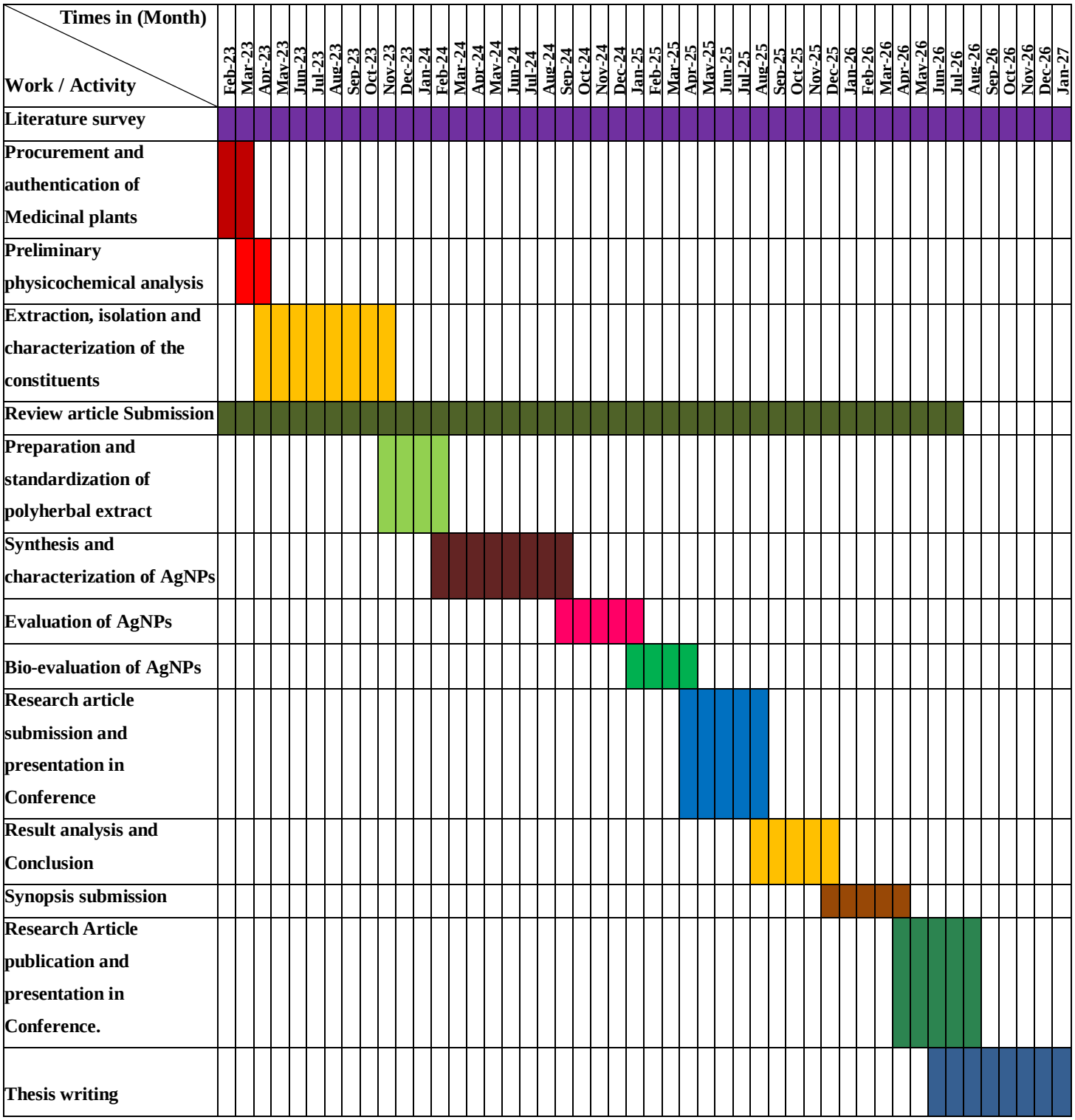
Group VI - Polyherbal extract + Isoproterenol

Group VII- Treatment – Silver nanoparticles loaded with Polyherbal extract + Isoproterenol

Groups IV to VII, will be administered test drug orally for 30 days. Standard orally administered feed + water will be given to all groups. Myocardial ischaemia will be produced in rats using isoproterenol subcutaneous injection on day 28^{nd} and 29^{th} at an interval of 24 hours in groups II to VI. Levels of cardiac biomarker CK-MB (Creatine kinase -Myocardial B fraction), Troponin I, LDH (Lactate dehydrogenase) will be studied in serum .Antioxidants– superoxide dismutase /reduced glutathione will be studied in serum. Blood samples of 12 hour fasted rats will be collected from retro orbital plexus at 0^{th}, 21^{st} and 30^{th} day to perform biochemical studies. ECG and blood pressure measurements will be done. After blood sampling on 30^{th} day, rats will be anaesthetized with anesthetic ether, then sacrificed and the hearts will be harvested for histological studies.

In treatment group, reduction in serum levels of cardiac biomarkers and increase in antioxidants will be seen as compared to disease group.

8. Statistical Plan



9. Limitations of the study

It is realized that the understanding of nanoparticle behavior is majorly dependent on the method of synthesis and characterization of the nanoparticles. Therefore the efforts have to be made for well-studied method of synthesis to produce well-defined particles, and their characterization for biological studies. Research studies reported reveal that it is very difficult to predict the exact amount of extract trapped inside the nanoparticle.

10. Expected outcomes of the study

As per the recent surveys, cardiovascular death is the leading cause of death worldwide, taking the lives of 18 million people each year.Hence, there is an urgent and unmet need to develop a novel, convenient, targeted and efficient approach for the treatment of CVDs. The proposed research study is a crucial step towards inspection of potential of traditional herbs for cardiovascular protection. This project will form a base of scientific data for more development of a herbal formulation already in market namely: Collasyn tablet manufactured by Dr. Palep's Laboratory. This study will have huge commercial importance. Thus the formulation prepared will betruly based on integrated principles of ethnobotany and modern day drug delivery systems. Previous studies have proved that several nano formulations could actually lead to cardio protection in patients diagnosed with CVDs. Also the herbs used in this research are well known for their cardio protective activity. These herbs also showed immense potential for management of COVID associated CVDs. Thus, loading the nano particles with these herbal extracts will not only reduce the repeated administration of drugs, even contribute towards overcoming patient non-compliance. It will also help in increasing the therapeutic value by reducing toxicity and increasing bioavailability.

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